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Associations of water quality and bacteria presence in cage cultured red hybrid tilapia, *Oreochromis niloticus* × *O. mossambicus*☆

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ABSTRACT

This study was conducted to understand the associations between water quality and the presence of bacteria in cage cultured red hybrid tilapia, *Oreochromis niloticus* × *O. mossambicus*. Tilapia from commercial floating net cage culture systems in lakes and river in Peninsular Malaysia were randomly sampled over a 24-month period. The eye, brain and kidney were sampled for bacterial isolation, following identification using biochemical test and commercial identification kits. The water clarity, velocity, depth, temperature, pH, iron, sulfide, ammonia, nitrite, phosphate, conductivity and dissolved oxygen at each sampling site were also measured. A total of 44 bacterial species were isolated, which are comprised of 28 Gram-positive and 16 Gram-negative bacteria. Terengganu River (506 isolates, 41 species) recorded the highest number of bacterial isolation and species, compared to Pedu Lake (286 isolates, 25 species) and Kenyir Lake (179 isolates, 25 species). The highest number of isolates was *Streptococcus agalactiae* (28.3%), followed by *Lactococcus lactis* (8.4%) and *Micrococcus* spp. (7.3%). Terengganu River had significantly lower ($P < 0.05$) water dissolved oxygen, temperature and pH compared to Pedu and Kenyir lakes. On the other hand, water iron, nitrite, sulfide, ammonia and velocity were significantly higher ($P < 0.05$) in Terengganu River compared to Pedu and Kenyir lakes. Multivariate analyses showed that each sampling site has different water quality parameters that were associated with the presence of bacteria. However, water temperature and ammonia have been identified as the most significant parameters, as they were observed to have strong associations with the bacteria presence in all of the study sites.

1. Introduction

Red tilapia (*Oreochromis* sp.) was the second highest harvested freshwater fish in Malaysia with a total production of 33,437 metric tons (MT) in 2013. The first and third highest were recorded for catfish (*Clarias* sp.) and river catfish (*Pangasianodon* sp.) with a total production of 50,534 MT and 13,914 MT, respectively. However, wholesale value of red tilapia was highest at RM 259 million, followed by catfish and river catfish, at RM 208 million and RM 138 million, respectively. These values significantly indicated that red tilapia farming is among the important aquaculture industry in this country. Recent analyses showed that the production of red tilapia were mainly from ponds (16,779 MT), followed by ex-mining pools (7668 MT) and freshwater cages (5518 MT) in rivers and lakes. It is also expected that the production of this fish will increase in the future due to commercialization of the industry (AFS, 2013).

Water quality is an important aspect in aquaculture system. Non-optimum water physico-chemical parameters (dissolved oxygen, pH, salinity, ammonia, temperature etc.) and poor management practices (overfeeding, inadequate nutrition, overcrowding etc.) can cause stress to the cultured fish and thus make them more susceptible to disease outbreaks (Boyd and Tucker, 1998; Zamri-Saad et al., 2014). Moreover, the intensiveness of fish culture industry and environment of the surrounding fish culture area also cause deleterious effects on the water quality (Gorlach-Lira et al., 2013).

☆ Statement of relevance: Understanding the associations between bacteria and their environment, especially water quality, helps in developing strategies for preventing or managing diseases caused by the potential fish pathogens.

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Several studies from various regions have been conducted to isolate pathogenic and non-pathogenic bacteria from cultured tilapia (Al-Harbi, 2003; Al-Harbi and Uddin, 2004; Marcel et al., 2013; Del'Duca et al., 2015). Various species of pathogenic bacteria for tilapia culture, such as *Vibrio* sp., *Flavobacterium* sp., *A. hydrophila* and *Streptococcus* sp., were isolated whether from the fish, water and sediment of the cultured tilapia (Al-Harbi and Uddin 2003, 2006). Even when some of the bacteria are microflora of the environment, they could cause epizootic diseases under environmental stress (Al-Harbi and Uddin, 2004).

Information on the influence of water quality on the presence of bacteria under actual field conditions is limited. Most of the previous studies solely characterized the bacteria that were isolated whether from water, sediment or different fish organs, while lacking in determination of the associations of the environmental variables, especially water quality in influencing the presence of the bacteria. Under true field conditions, as experienced with real fish culture in natural water bodies, combinations of various environmental variables, especially from the water quality may influence the presence of bacteria (Arulampalam et al., 1998; Vezzulli et al., 2002). Thus, this study was conducted in order to determine the associations between water quality and the presence of bacteria in cage cultured red hybrid tilapia, *Oreochromis niloticus* × *O. mossambicus*, in selected lakes and river in Peninsular Malaysia.

2. Materials and methods

2.1. Sampling sites

This study was conducted in two types of water bodies, the lake and river. Study involving lake consisted of two sampling sites, the Pedu Lake in Kedah and Kenyir Lake in Terengganu. Sampling in river was conducted along Terengganu River, Terengganu, which include samples collected from Kuala Kejir, Pantai Ali, Beladau Selat and Beladau Kepong. Kuala Kejir is situated at the upper stream, followed by Pantai Ali, Beladau Selat and Beladau Kepong (Fig. 1).

The lakes were extremely big with very slow to no water flow, while for river, the size was moderate with modest to fast flowing water. Pedu and Kenyir lakes are used as irrigation for paddy field and hydroelectric, respectively, and are located approximately 10 km away from residential areas. Terengganu River originated from Kenyir Lake and flows through sand dredging, agricultural and residential areas. More than 200 floating net cages were present at the sampling sites in Pedu and Kenyir lakes but less than 100 floating net cages were present at each sampling sites along the Terengganu River.

The fish used in this study were owned by private farmers and commercially cultured in floating net cages culture system. When the fish were harvested, the farmers introduced new batch of fish, making the fish culture continuous throughout the year with different batches of sizes, ages, and sources of fingerlings at any one time. The fish were cultured for 6–8 months to reach marketable size, which was more than 500 g. The fish stocking density was between 1000–1500 fish per cage, depending of the size of the cages. All fish were fed with commercial fish pellet twice a day.

2.2. Fish sampling

This study was conducted for a period of 24 months, with monthly intervals of sampling. A total of 23–74 fish were randomly collected from different cages at each sampling site, which included various batches of fish with different sources of fingerlings, sizes and age. Cumulatively, a total of 719 fish were sampled from Pedu Lake, 1010 from Kenyir Lake, 679 from Beladau Selat, 606 from Beladau Kepong, 711 from Pantai Ali and 614 from Kuala Kejir during the study period.

2.3. Isolation and identification of bacteria from fish

Briefly, swabs from individual fish were taken from the eye, brain and kidney, and streaked directly onto Tryptic Soy Agar (Merck, Darmstadt, Germany) with 5% human blood. The agars were then incubated for 24 h at 37 °C. The fish were handled and sacrificed according to method approved by Animal Utilization Protocol, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

The identification procedures of various isolated bacteria from the fish were referred to Al-Harbi and Uddin (2005). Briefly, the grown bacteria were sub-cultured to get the pure colonies. The bacterial colonies isolated were then divided into different types according to the colony characteristics of size, structure, shape, elevation, edge, surface, opacity and color. All of the pure isolates were also determined for their Gram staining, cell shape and motility. The isolates were then subjected to biochemical tests such as oxidase, catalase, amylase, gelatinase, lipase, indole, H_2S production, nitrite reduction, etc. as described in Bergey's Manual of Determinative Bacteriology for identification to genus or species level (Holt et al., 1994). The presumptive *Vibrio* species were confirmed by their growth in different thiosulphate-citrate-bile sucrose (TCBS) agar (Difco™, New Jersey, USA), followed by their sensitivity test to the vibriostatic agent (0/129) (Bio-Rad, California, USA). The isolates characterized as Gram-positive cocci and catalase-negative were also then identified to species level using API® 20 Strep (bioMérieux, Marcy l'Etoile, France); Gram-positive cocci and catalase-positive were identified using API® Staph (bioMérieux); and Gram-negative rod were identified using API® 20E (bioMérieux).

The bacterium was considered present in an individual fish when isolated from at least one of the organs (eye, brain and kidney), and more than one species of bacteria might be present in an individual fish. Due to the large number of isolated bacteria and for the accuracy of the results interpretation, only bacteria that were present more than once in each sampling sites were further analyzed to determine their relationship with water quality parameters.

2.4. Water quality sampling and measurement

Water quality at 1 m depth were measured from two consistent sampling points surrounding and within the fish cages, respectively. Similar with fish sampling, the measurement were made at monthly intervals for 24 months of the study period, after the fish sampling activities.

Water quality parameters were measured *in situ* using a handheld YSI meter (YSI, Yellow Springs, OH, USA), including pH, temperature and dissolved oxygen. The concentration of water nitrite, sulfide and ammonia were then determined using powder pillow procedures and the concentration measurement were made using a spectrophotometer (HACH Company, Loveland, CO, USA).

The water flow rate, depth and clarity were measured using a current water meter (Global Water, Gold River, CA, USA), ultrasonic depth sensor (Speedtech Instrument, Logan, UT, USA) and Secchi disk, respectively.

The monthly recorded data of water quality were used in order to identify possible associations with the monthly presence of bacteria species, while the mean ± standard deviation (SD) of water quality throughout the study period were calculated.

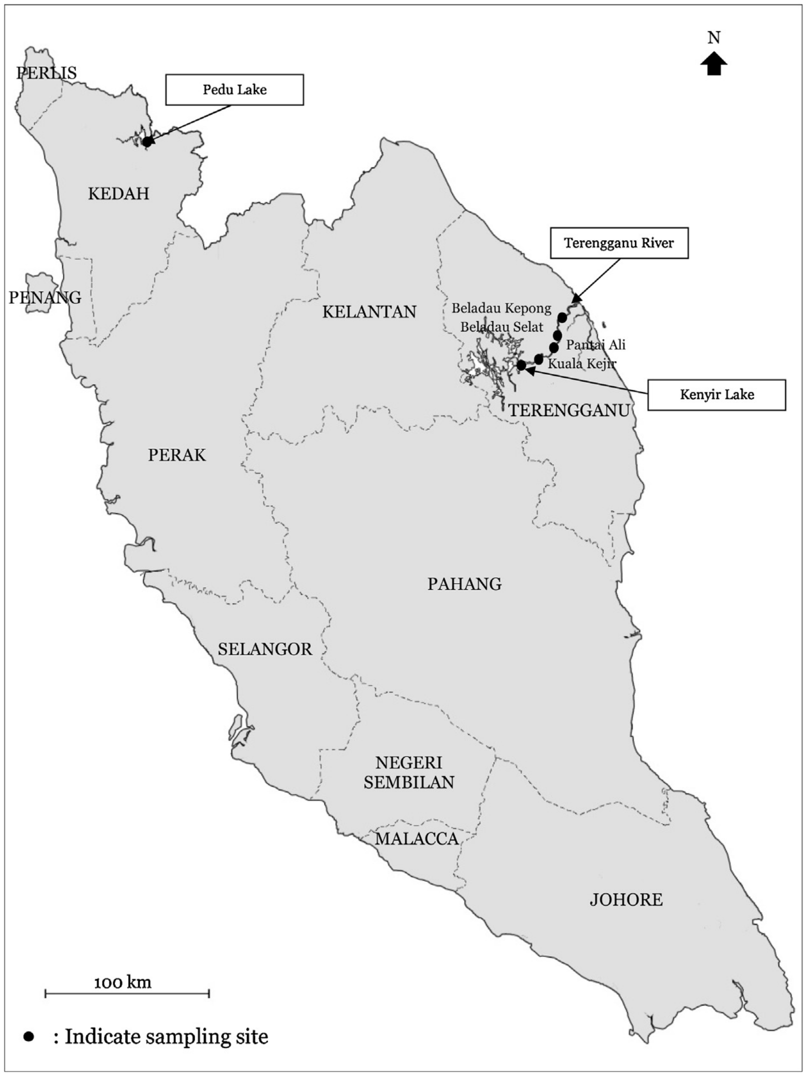

Fig. 1. Location of Pedu Lake, Kenyir Lake and sampling sites along Terengganu River, Peninsular Malaysia.

2.5. Statistical analysis

The number of bacteria presence and their percentage during the 24 months study period were calculated based on the sampling sites, while the mean ± SD of water quality were measured and compared between each sampling site using the analysis of variance (ANOVA) with Tukey LSD All-Pairwise Comparison Test (Statistix 9, Analytical Software, Tallahassee, FL, USA). A P value at <0.05 was used to indicate statistical significance.

A data matrix was then constructed in Microsoft Excel spreadsheet (Microsoft Corporation) which comprised of all isolated bacteria and their number of presence in each sampling time, and all water quality measurement data. For better understanding of the results, all four sampling sites along the Terengganu River were combined and their mean ± SD data were used for further analyses. The data matrix was processed for analyses and prepared separately each for Pedu Lake, Kenyir Lake and Terengganu River.

Next, to identify the associations between water quality and the presence of bacteria, with the exception of water clarity, flow and depth, nine water quality parameters were taken into consideration including temperature, pH, iron, sulfide, ammonia, nitrite, phosphate, conductivity and dissolved oxygen.

Dataset comprising of numerous variables is likely quite redundant if two or more variables have a high correlation to each other. Principal components analysis (PCA) using SPSS (IBM SPSS Statistics Version 22) was performed to reduce the number of variables in a dataset while retaining the variability in the data. By using PCA, the variables that are correlated with one another were combined into factors. Factor loadings greater than 0.30 in absolute value are considered to be significant (Tabachnick and Fidell, 2007).

The relationship between water quality with the presence of bacteria were then analyzed using canonical correspondence analysis (CCA). The CCA was examined using XLSTAT add-in for Microsoft Excel. For this analysis, only parameters that showed variation in their values in each sampling sites were used. The significance of each variable was tested using CCA in XLSTAT-ADA with 5000 permutations at a significance level of 5%. Results are presented using canonical correspondence analysis biplots and other descriptive statistics.

3. Results

3.1. Bacteria isolation and identification

A total of 44 bacterial species were isolated; 28 were Gram-positive and 16 were Gram-negative bacteria. Terengganu River recorded the highest number of bacterial isolation with 506 isolates, followed by Pedu Lake with 286 isolates and Kenyir Lake with

Table 1
List of bacteria, abbreviation used, their number and percentage from Kenyir Lake, Pedu Lake and Terengganu River.

Bacteria	Abbreviation	Kenyir Lake (n = 179)		Pedu Lake (n = 286)		Terengganu River (n = 506)	
		Number	%	Number	%	Number	%
Acinetobacter baumannii	A.bau					10	2.0
[a]*Aerococcus viridans*	A.vir					6	1.2
Aeromonas hydrophila	A.hyd	12	6.7	9	3.1	4	0.8
Edwardsiella tarda	E.tar					13	2.6
Enterobacter cloacae	E.clo	2	1.1			5	1.0
[a]*Enterococcus faecalis*	E.lis					2	0.4
[a]*Enterococcus faecium*	E.ium			11	3.8	20	4.0
Escherichia coli	E.col			3	1.0		
[a]*Gemella haemolysans*	G.hae					2	0.4
[a]*Kocuria varians*	K.var	5	2.8	8	2.8	40	7.9
[a]*Lactococcus lactis*	L.lac	4	2.2	27	9.4	51	10.1
[a]*Leuconostoc* spp.	Leuc			10	3.5	16	3.2
[a]*Micrococcus* spp.	Micr	18	10.1	13	4.5	40	7.9
Morganella morganii	M.mor					3	0.6
Ochrobactrum anthropi	O.ant					7	1.4
Pantoea spp.	Pant	4	2.2	5	1.7	6	1.2
Pasteurella haemolytica	P.hae			3	1.0	5	1.0
Pasteurella spp.	Past			2	0.7		
Plesiomonas shigelloides	P.shi	3	1.7	7	2.4	13	2.6
Proteus mirabilis	P.mir					2	0.4
Proteus vulgaris	P.vul					6	1.2
Pseudomonas aeruginosa	P.aer					9	1.8
Salmonella spp.	Salm					2	0.4
[a]*Staphylococcus aureus*	S.aur	12	6.7	6	2.1	10	2.0
[a]*Staphylococcus auricularis*	S.auri	1	0.6	2	0.7	2	0.4
[a]*Staphylococcus capitis*	S.cap	4	2.2	4	1.4	14	2.8
[a]*Staphylococcus caprae*	S.capr			1	0.3	2	0.4
[a]*Staphylococcus cohnii*	S.coh	3	1.7	4	1.4	13	2.6
[a]*Staphylococcus epidermidis*	S.epi	2	1.1	3	1.0	5	1.0
[a]*Staphylococcus haemolyticus*	S.hae	4	2.2			4	0.8
[a]*Staphylococcus hominis*	S.hom	4	2.2	3	1.0	8	1.6
[a]*Staphylococcus lentus*	S.len	1	0.6	6	2.1	16	3.2
[a]*Staphylococcus schleiferi*	S.sch	1	0.6			3	0.6
[a]*Staphylococcus sciuri*	S.sci	5	2.8	6	2.1	30	5.9
[a]*Staphylococcus* spp.	Stap	6	3.4			12	2.4
[a]*Staphylococcus warneri*	S.war	3	1.7			6	1.2
[a]*Staphylococcus xylosus*	S.xyl	11	6.1	18	6.3	16	3.2
Stenotrophomonas maltophilia	S.mal					11	2.2
[a]*Streptococcus acidominimus*	S.aci	1	0.6	2	0.7	2	0.4
[a]*Streptococcus agalactiae*	S.aga	69	38.5	130	45.5	76	15.0
[a]*Streptococcus constellatus*	S.con	1	0.6	1	0.3		
[a]*Streptococcus dysgalactiae*	S.dys	1	0.6			6	1.2
[a]*Streptococcus suis*	S.sui					3	0.6
[a]*Streptococcus uberis*	S.ube	2	1.1	2	0.7	5	1.0

[a] Indicate Gram-positive bacteria.

179 isolates. Isolates from Terengganu River were from 41 different species of bacteria compared to 25 different bacteria species recorded in Kenyir and Pedu lakes, respectively. The three highest bacteria species isolated were *Streptococcus agalactiae* (275 isolates; 28.3%), *Lactococcus lactis* (82 isolates; 8.4%) and *Micrococcus* spp. (71 isolates; 7.3%). The bacteria species with lowest presence (2 isolates; 0.2%) were *Enterococcus faecalis*, *Gemella haemolysans*, *Pasteurella* spp., *Proteus mirabilis*, *Salmonella* spp. and *S. constellatus*. The details on the isolated bacteria, their number and percentages in each sampling sites are presented in Table 1.

3.2. Water quality measurement

The mean ± SD of water quality parameters recorded in Pedu Lake, Kenyir Lake and Terengganu River are presented in Table 2. Water dissolved oxygen ($7.57 \pm 0.94\ \mathrm{mg\,L^{-1}}$) and pH ($7.53 \pm 0.74$) was highest in Pedu Lake, but this was not significantly different ($P > 0.05$) from the readings measured in Kenyir Lake. The water temperature measured in Pedu Lake ($29.56 \pm 1.29\ ^{\circ}\mathrm{C}$) and Kenyir Lake ($29.56 \pm 1.42\ ^{\circ}\mathrm{C}$) showed almost similar readings, while water conductivity ($53.00 \pm 19.31\ \mu\mathrm{S\,cm^{-1}}$) and phosphate ($0.030 \pm 0.063\ \mathrm{mg\,L^{-1}}$) were significantly ($P < 0.05$) highest and lowest, respectively, in Pedu Lake compared to other sampling sites. The water clarity (425 ± 70 cm) and depth of sampling sites (69.18 ± 7.45 m) were significantly highest ($P < 0.05$) in Kenyir Lake, compared to Pedu Lake and Terengganu River.

Water quality readings in Terengganu River were significantly lowest ($P < 0.05$) for dissolved oxygen ($5.55 \pm 0.95\ \mathrm{mg\,L^{-1}}$), temperature ($27.22 \pm 1.42\ ^{\circ}\mathrm{C}$) and pH ($6.33 \pm 0.71$), but significantly highest ($P < 0.05$) for iron ($0.795 \pm 0.326\ \mathrm{mg\,L^{-1}}$), nitrite ($0.00852 \pm 0.00879\ \mathrm{mg\,L^{-1}}$), sulfide ($9.67 \pm 7.77\ \mathrm{mg\,L^{-1}}$), ammonia ($0.243 \pm 0.091\ \mathrm{mg\,L^{-1}}$) and velocity ($0.251 \pm 0.367\ \mathrm{cm\,s^{-1}}$), compared to Pedu and Kenyir lakes.

3.3. Principal component analysis of water quality

Principal component loadings from principal component analysis of water quality parameters from Pedu Lake, Kenyir Lake and Terengganu River are presented in Table 3. For each sampling site, the PCA produced two axes that cumulatively explained 72.69%, 68.16% and 68.17% of water quality variations in Pedu Lake, Kenyir Lake and Terengganu River, respectively. From nine water quality parameters evaluated, only five parameters were retained in each sampling site, which were temperature, pH, iron, sulfide and

Table 2
Water quality parameters recorded in Pedu Lake, Kenyir Lake and Terengganu River.

Parameters	Pedu Lake		Kenyir Lake		Terengganu River	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Dissolved oxygen (mg L^{-1})	7.57 ± 0.94^a	5.61–9.40	7.38 ± 0.73^a	6.25–9.12	5.55 ± 0.95^b	3.05–9.15
Temperature (°C)	29.56 ± 1.29^a	27.00–31.30	29.56 ± 1.42^a	26.70–31.33	27.22 ± 1.42^b	23.70–30.97
pH (1–14)	7.53 ± 0.74^a	5.83–8.61	7.21 ± 0.42^a	6.15–7.87	6.33 ± 0.71^b	4.00–8.01
Iron (mg L^{-1})	0.093 ± 0.061^b	0.010–0.313	0.060 ± 0.034^b	0.010–0.160	0.795 ± 0.326^a	0.220–1.720
Conductivity (μS cm^{-1})	53.00 ± 19.31^a	44.67–138.00	29.00 ± 3.16^b	23.25–34.25	30.86 ± 9.76^b	22.00–68.00
Nitrite (mg L^{-1})	0.004 ± 0.005^b	0.000–0.024	0.002 ± 0.001^b	0.000–0.006	0.009 ± 0.009^a	0.000–0.053
Sulfide (mg L^{-1})	3.60 ± 1.96^b	0.00–9.00	2.75 ± 1.59^b	1.25–7.57	9.67 ± 7.77^a	0.00–36.00
Ammonia (mg L^{-1})	0.062 ± 0.054^b	0.000–0.240	0.019 ± 0.007^b	0.003–0.038	0.243 ± 0.091^a	0.045–0.490
Phosphate (mg L^{-1})	0.030 ± 0.063^b	0.000–0.276	0.108 ± 0.081^a	0.000–0.277	0.110 ± 0.143^a	0.000–1.060
Secchi disc (cm)	243 ± 40^b	173–300	425 ± 70^a	263–515	86 ± 44^c	10–240
Velocity (cm s^{-1})	0.009 ± 0.015^b	0.000–0.027	0.005 ± 0.008^b	0.000–0.015	0.251 ± 0.367^a	0.000–2.122
Depth (m)	20.50 ± 3.00^b	17.00–27.70	69.18 ± 7.45^a	62.00–76.70	3.21 ± 1.00^b	0.93–5.63

[a,b,c]Different letters represent significant differences ($P < 0.05$) between the same row for mean ± SD measurements only.

Table 3
Principal component loadings from principal component analysis of water quality parameters from Pedu Lake, Kenyir Lake and Terengganu River.

Parameters	Pedu Lake		Kenyir Lake		Terengganu River	
	PC1	PC2	PC1	PC2	PC1	PC2
Percentage variance explained	44.942	27.750	46.454	21.708	47.871	20.303
Cumulative variance explained	44.942	72.692	46.454	68.163	47.871	68.174
Eigenvalue	1.798	1.110	2.323	1.085	2.394	1.015
Temperature	0.828	0.238	0.722	0.208	0.061	**0.734**
pH	**0.865**	−0.038				
Iron	**0.316**	**0.752**			**0.823**	0.119
Sulfide	−0.079	**0.863**	**0.351**	**0.829**		
Ammonia	0.243	**0.718**	**0.766**	−0.078	**0.847**	0.169
Nitrite			**0.701**	**0.356**		
Phosphate			−0.015	**0.906**		
Conductivity					**0.861**	0.023
Dissolved oxygen					−0.164	**−0.834**

Water quality loadings with absolute value >0.30 in bold.

ammonia in Pedu Lake, temperature, sulfide, ammonia, nitrite and phosphate in Kenyir Lake, and temperature, iron, ammonia, conductivity and dissolved oxygen in Terengganu River.

For Pedu Lake, the first axis had high loadings of water temperature, pH and iron, while the second axis had high loadings of iron, sulfide and ammonia. However for Kenyir Lake, the water temperature, sulfide, ammonia and nitrite were observed to show high loadings on the first axis, but sulfide, nitrite and phosphate for the second axis. The measured water iron, ammonia and conductivity showed high loadings on the first axis, while temperature and dissolved oxygen on the second axis in Terengganu River.

3.4. Canonical correspondence analysis of water quality

In each sampling site, CCA resulted in the retention of all five water quality variables from the PCA as significant contributors to the variation in the ordination. Moreover, five ordination axes were generated for each CCA in each sampling site, while cumulative percentage for the first and second ordination axes for Pedu Lake, Kenyir Lake and Terengganu River were observed at 56.34%, 59.94% and 57.83%, respectively (Table 4).

For Pedu Lake, with respect to the first and second axes only, both axes were positively correlated with water temperature and iron, but were negatively correlated with pH. Water ammonia was negatively correlated with first axis, but positively correlated with second axis. This observation, however, was vice versa for sulfide. While in Kenyir Lake, both axes showed positive association with nitrite and phosphate, but negatively with sulfide. Water temperature and ammonia showed positive relationship with the first and second axis, respectively. As for Terengganu River, only dissolved oxygen showed negative relationships with both axes, while the other parameters showed whether positive or negative correlation in the first or second axis.

3.5. Relationship between the presence of bacteria and water quality

Fig. 2 shows the CCA ordination diagram on the effect of water quality on the presence of 25 bacteria species in Kenyir Lake. The longest CCA vector was observed for water temperature, followed by nitrite, phosphate, sulfide and ammonia. Briefly, several species of bacteria such as *S. acidominimus*, *S. constellatus*, *Plesiomonas shigelloides* and *S. agalactiae* showed associations with high water temperature, but the opposite was observed for species such as *Staphylococcus cohnii*, *S. capitis*, *S. sciuri* and *Enterobacter cloacae*. Regarding phosphate, bacteria species such as *Pantoea* spp. and *S. dysgalactiae* showed positive relationships compared to *S. sciuri* and *S. warneri*, which showed negative relationships, while the sulfide reading clearly showed negative relationships with phosphate and temperature. Besides that, bacteria species such as *S. epidermidis*, *S. hominis* and *S. lentus* increased in high ammonia environment, while bacteria like *Kocuria varians*, *S. aureus* and *S. xylosus* showed a negative association with ammonia.

The CCA ordination diagram on the effect of water quality on the presence of 25 bacteria species in Pedu Lake are presented in Fig. 3. Similar with Kenyir Lake, the longest CCA vector was observed for water temperature, followed by pH, ammonia, sulfide and iron. Several bacteria species, namely *L. lactis*, *E. faecium*, *S. aureus* and *S. xylosus* showed positive relationships with increased water temperature, but was negatively correlated with species of bacteria such as *Aeromonas hydrophila*, *S. auricularis*, *Pasteurella haemolytica* and *S. sciuri*. However, for the presence of *S. lentus* and *S. xylosus*,

Table 4
Canonical correspondence analysis summary statistics for water quality parameters at Pedu Lake, Kenyir Lake and Terengganu River.

Parameters	Pedu Lake					Kenyir Lake					Terengganu River				
	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5
Eigenvalue	0.364	0.334	0.277	0.142	0.121	0.446	0.209	0.195	0.168	0.127	0.226	0.192	0.134	0.114	0.056
Cumulative%	29.38	56.34	78.73	90.22	100.00	38.59	59.94	74.28	88.96	100.00	31.28	57.83	76.42	92.26	100.00
Regression coefficient															
Temperature	1.054	0.521	0.010	−1.116	−0.366	1.106	−0.016	0.319	0.003	0.120	0.532	−0.474	−0.519	−0.633	0.403
pH	−0.386	−0.765	−1.151	0.391	0.403										
Iron	0.044	0.078	0.957	0.967	0.932						0.026	−0.522	−0.478	1.033	0.235
Ammonia	−0.258	1.000	−0.127	−0.139	0.272	−0.172	0.160	0.722	−0.066	−0.696	−0.188	1.216	−0.496	−0.008	0.194
Sulfide	0.404	−0.186	−0.992	0.265	−0.950	−0.285	−0.101	−0.830	0.925	−0.690					
Nitrite						0.033	0.867	−0.327	−0.678	0.133					
Phosphate						0.289	0.349	1.046	0.302	0.749					
Conductivity											0.557	−0.326	0.353	−0.105	−1.071
Dissolved oxygen											−0.410	−0.541	−0.960	−0.015	−0.256

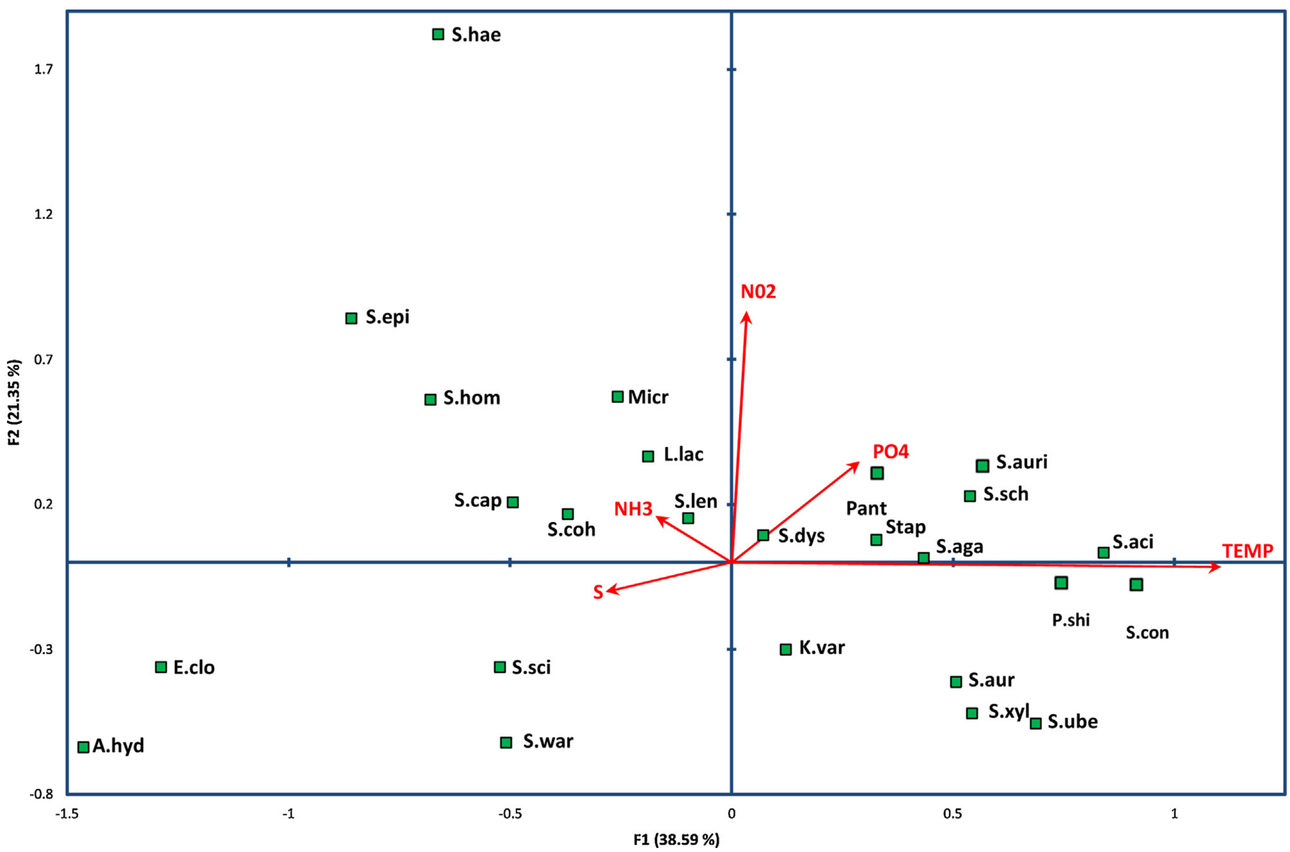

Fig. 2. Canonical correspondence analysis ordination diagram showing the effect of water quality parameters on the presence of bacteria in Kenyir Lake.

they were inversely related with increased pH, but were positively associated with increase in iron. The results also showed that the presence of *S. agalactiae*, *S. uberis* and *Pantoea* spp. were negatively correlated with the increased in ammonia, while the presence of *P. shigelloides*, *S. cohnii* and *Micrococcus* spp. were negatively associated with the increase in sulfide.

Canonical correspondence analysis ordination diagram showing the effect of water quality on the presence of 44 bacterial species in Terengganu River are presented in Fig. 4. For this sampling site, the longest CCA vector was observed for water ammonia, followed by temperature, conductivity, dissolved oxygen and iron. Most bacteria gathered at the center of the diagram, which was different from Pedu and Kenyir lakes where the bacteria were more scattered. Water ammonia showed negative associations with the presence of bacteria such as *P. shigelloides, Staphylococcus* spp., *S. haemolyticus* and *Stenotrophomonas maltophilia*. Similarly, iron showed negative correlations with the increase in ammonia and several bacteria species such as *S. uberis, S. dysgalactiae*, *Aerococcus viridans* and *S. acidominimus*. Water temperature and conductivity also showed negative associations with the presence of bacteria such as *S. caprae*, *E. cloacae* and *S. warneri*, but was positively associated with *S. auricularis, K. varians* and *E. faecium*. The presence of several bacteria species such as *P. haemolytica*, *A. hydrophila*, *S. hominis* and *Morganella morganii* were positively associated with higher dissolved oxygen, but negatively associated with *S. capitis, S. lentus, S. epidermidis* and *S. agalactiae*.

4. Discussion

This study revealed the associations of water quality parameters with the presence of various bacterial species in cage cultured red hybrid tilapia at several major aquaculture production sites in

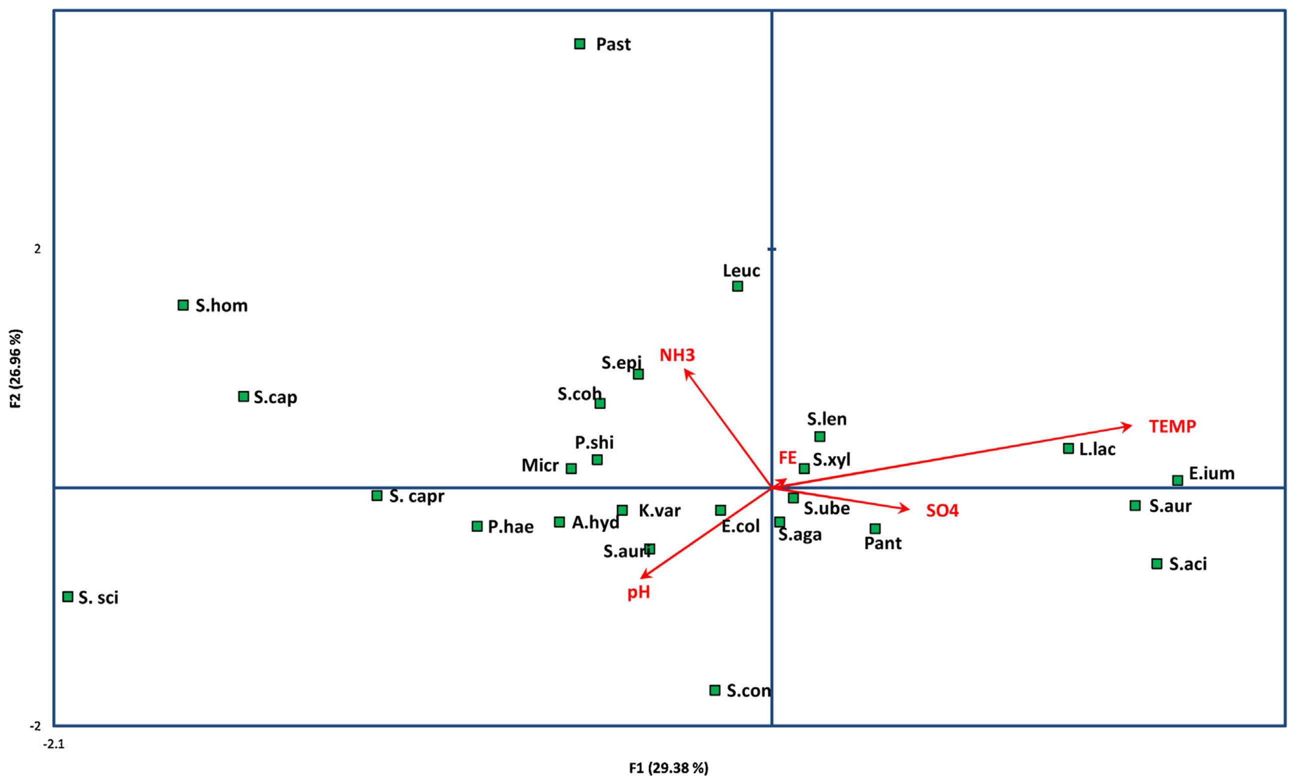

Fig. 3. Canonical correspondence analysis ordination diagram showing the effect of water quality parameters on the presence of bacteria in Pedu Lake.

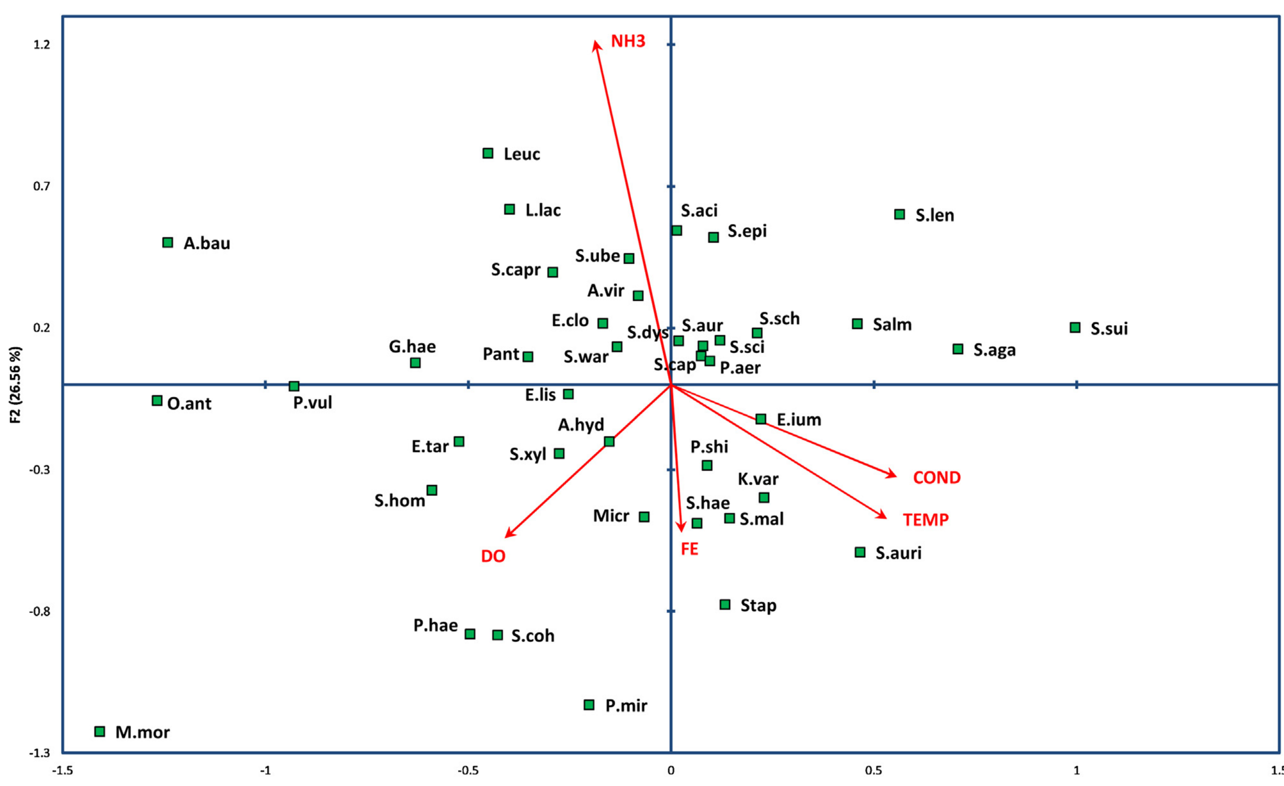

Fig. 4. Canonical correspondence analysis ordination diagram showing the effect of water quality parameters on the presence of bacteria in Terengganu River.

Peninsular Malaysia. The findings showed that some of the water quality parameters, such as water pH (low) and ammonia (high) in Terengganu River were not in the recommended range for tilapia culture (Zamri-Saad et al., 2014). Moreover, the water temperature, which is an important pre-disposing factor for bacterial diseases in fish (Amal et al., 2010b; Amal and Zamri-Saad, 2011; Zhang et al., 2016), were quite high in Pedu and Kenyir lakes. Most water quality measurements in Terengganu River were significantly different from Pedu and Kenyir lakes, which may be due to the surrounding activities particularly the agricultural, residential area and sand dredging activities that affected the water quality (Lee et al., 2010).

Most of the bacteria (52.11%) isolated from this study were obtained from Terengganu River, compared to Pedu Lake (29.5%) and Kenyir Lake (18.4%). Factors such as agricultural and domestic sewages that have been discharged into the river may increase the organic contents of the river water and promote the growth of various bacteria species, as supported by a study in the same river by Lee et al. (2010). A wide range of bacterial taxa also has been recovered from red tilapia in this study, similar to previous reports in this country and other different region (Al-Harbi and Uddin, 2003, 2005, 2007; Amal et al., 2010a; Marcel et al., 2013). However, the dominant bacteria isolated from the cultured tilapia were Gram-positive (63.6%), as similarly observed in Kenyir Lake, Terengganu and Semantan River, Pahang in Malaysia (Marcel et al., 2013), but was contrary with those reported from ponds and brackish water area in Saudi Arabia (Al-Harbi and Uddin, 2003, 2005).

Several bacterial species identified in this study were pathogenic to fish, which included *A. hydrophila, Edwardsiella tarda, P. aeruginosa, S. dysgalactiae* and *S. agalactiae* (Leung et al., 1995; Suanyuk et al., 2008; Lee et al., 2011; Netto et al., 2011; Shayo et al., 2012; Anshary et al., 2014), while species such as *Bacillus* sp., *Micrococcus* spp., *P. aeruginosa*, *L. lactis* and *E. faecalis* have been developed into probiotics in aquaculture (Apún-Molina et al., 2009; El-Rhman et al., 2009; Allameh et al., 2014; Priyaja et al., 2014; Beck et al., 2015). Few species were reported harmful to humans such as *Acinetobacter baumannii, Escherichia coli, M. morganii, S. aureus* and *Salmonella* spp. (Jarraud et al., 2002; Gläser et al., 2004; Mermin et al., 2004; Falagas et al., 2006; McConnell et al., 2013) and animals such as *M. morganii*, *Salmonella* spp. and *Pasteurella* spp. (Mermin et al., 2004; Zhao et al., 2012; Annas et al., 2014). Marcel et al. (2013) isolated more than one species of bacteria from a single fish, and they believed that this is associated with the nature of feed given to the fish, location of sampling sites, nearby human activities and water quality at the fish culture area, which is similarly observed in our findings. Moreover, most of the isolated bacteria in this study have also been isolated from the cage cultured tilapia in this country (Siti-Zahrah et al., 2004, 2008; Amal et al., 2010a).

Environmental conditions surrounding the culture sites may affect the water quality and stress the cultured fish. This eventually decreases the immune status, triggering bacterial infection leading to disease outbreaks (Amal et al., 2015). The bacterial flora of the fish also reflects the bacterial compositions of the current fish cultured environments (water and sediment) and fish health status (Al-Harbi and Uddin, 2003; Pakingking et al., 2015). However, according to Irianto and Austin, (2002), bacteria in fish can have mutualistic and pathogenic associations with their host. The significant roles of fish microbiota are to protect the host against pathogenic challenge by production of antagonistic factors, inactivation of pathogenic bacterial toxins or metabolites, stimulation of host immunity and competition with pathogens for attachment sites or nutrients. All of these information may provide an explanation for our finding on why so many species, whether pathogenic and non-pathogenic, were present in the cultured fish.

Multivariate analyses showed that water temperature, ammonia, iron, sulfide, nitrite, phosphate, pH, dissolved oxygen and conductivity were important in at least one of the study site, while each sampling site has different water quality parameters that were associated with the presence of bacteria. All measured water quality parameters showed their importance in influencing the occurrence of the bacteria. Water quality have been identified and well discussed as important factors in influencing the presence of non-pathogenic or pathogenic fish bacteria and also in bacterial community in fish cultured environments (Walters and Plumb, 1980; Ortega et al., 1996; Arulampalam et al., 1998; El-Shafai et al., 2004; Amal et al., 2015). Moreover, the poor and non-optimum water quality may have induced weakness and stress to the fish, resulting in a greater susceptibility to bacterial infections (Escher et al., 1999).

Physico-chemical parameters of the water, nutrients and presence of toxic compounds may influence the density of bacterial populations (Gorlach-Lira et al., 2013). This study revealed that water temperature and ammonia influenced the presence of various bacteria at all sampling sites. Similarly, Rheinheimer (1985) found that high water temperature is an optimum condition for various mesophilic bacteria to grow, thus play an important role in influencing their presence in cultured fish. Moreover, in a tropical country like Malaysia, water temperature plays significant role in the dynamics of nutrient, which is interrelated with other water quality parameters (Gorlach-Lira et al., 2013). The ammonia in water from lakes is believed to originate from the fish feces and excessive feed given to the fish. However in Terengganu River, the various activities along the river may have also increased the ammonia concentration in the river, as observed in our study.

5. Conclusion

This study revealed that various pathogenic and non-pathogenic bacteria were isolated from the cage cultured red hybrid tilapia. We also identified the significant water quality parameters that influenced the presence of the bacteria in cultured fish. Even though each sampling site has different water quality parameters that were associated with the presence of bacteria, water temperature and ammonia have been identified as the most significant parameters as they were observed to have strong associations with the presence of bacteria in all of the sampling sites. Understanding the association between bacteria and their environment, especially water quality, will help us develop strategies for preventing or managing diseases caused by the potential fish pathogens in the future.

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